Multiple Regeneration of *Clinacanthusnutans* Nodal Explants by using 6-Benzylaminopurine (BAP) Hormone

Siti Zulaiha Ghazali[1], Saiyidah Nafisah Hashim[2]
[1]School of Biology, Faculty of Applied Science, Universiti Teknologi MARA (Perlis), Malaysia,
Email: ctzulaiha_91@yahoo.com
[2]Applied Science Department, Faculty of Applied Science, UniversitiTeknologi MARA (Perak), Malaysia,
Email: saiyidah394@perak.uitm.edu.my

Abstract

Clinacanthusnutans is a medicinal plants currently found to be able to treat cancer. This plant can be found mostly in China, Thailand and Malaysia. Since a large portion of pharmaceutical drugs are derived, the demand for these raw materials is steadily rising. In order to fulfill the demand on the numbers of *C. nutans* plant in the drug production, multiple regenerations by plant tissue culture technique is applied and production of undifferentiated callus were done. Culture environmental condition was controlled and sterile nodal explants were inoculated in a MurashigeSkoog (MS) media supplemented with plant hormone cytokinin, BAP (6 – Benzylaminopurine) at different concentrations (0 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L). Plants were sub-cultured at every 4 weeks and sterile grown plants were used asexplants to grow highly proliferated callus at different media concentration. Callus inductions were also tested at two different time period. The observation of the regeneration was observed and analyzed after 8 weeks. Results showed MS media with 2.0 mg/L BAP gave the highest regeneration, mean number leaves and height of explants formation with 11.20 ± 0.374 and 5.260 ± 0.404 respectively. Besides that, at 2.0 mg/L concentration, the explants show the formation of roots after 7 weeks. The formulated media in regenerating this valuable herbal plant with anticancer beneficiary compound will give a significant contribution to the society.

Keywords:Clinacanthusnutans, anticancer, 6– Benzylaminopurine, regeneration, nodal

1.0 Introduction

Therapeutic drug studies on medicinal plants had gained great attention from both developing and advanced countries across the world (Veeresham and Chitti, 2013). *Clinacanthusnutans* is one of the famous medicinal plants categorized as tropical herbs and has been widely used in Southern Asia including Malaysia, China and Thailand and had been used for medicinal purpose (Ying, 2013).A tissue culture technique has been applied to produce a massive healthy of new plantlets due to increasing demand of medicinal plants. The first plant tissue culture that established by Gautheret was obtained from the cambial tissue of *Acer pseudoplatanus*, *Ulmuscmpestre*and*Salix capraea*by using agar-solidified medium Knop's solution plus glucose and cystein hydrochloride (Thorpe, 2005).Suitable surface sterilization technique should be established and accurate concentration of hormones must be clarified with suitable environment condition to regenerate the plantlets. However, in order to produce the whole plant through *in vitro* technique, the primary sources of the whole plant must be in a good condition. This study focused on to seek the appropriate concentration of plant hormone in mass propagating of multiple numbers of shoots through plant regeneration process. It also provides efficiency protocol for mass production of *C. nutans* for continuous supply. The *in vitro* technique for plant regeneration will offer the continuous supply of healthy and high quality sterile plantlets for medicinal production with limited time consuming. Besides that, it willcontribute towards important findings on optimal BAP concentration needed in shooting regeneration of *C. nutans.*Micropropagation is the proven method for efficiency in vitro propagation of medicinal and aromatic plants and for commercial exploitation of valuable plant-derived pharmaceutical (Purkayastha et al., 2007).Thus, the objective of the study is to regenerate shoot from sterile nodal explants of *C. nutans*in different concentration of BAP.

2.0 Material and Methods

The stem of the *C. nutans* was obtained from plant nursery at Arau, Perlis. Murashige and Skoog (MS)medium. 3% of sucrose was added into distilled water where it acts as main carbon sources for the medium. 2.2 g and 0.05 g of MS powder and myo-inositol was added to the medium and was dissolved on the hot plate. The pH was adjusted by using 1 M NaCl or 1 M NaOHforpH 5.8 ± 0.1. 2.2 g gelrite was added to solidify the medium. The medium was autoclaved under 15 lbs psi for 15 minutes at 121°C.

2.1 Surface sterilization

Plantnodal part was excised about 1 cmfromthe stem and surface sterilizationwascarried out. The nodal explants were placed into 10% commercial Clorox for 10 minutes. Explants were thenrinsed 3 times with distilled water and dipped into the fungicide of mercury chloride for 1 hour at 0.1 % (w/v). Next, the nodal were rinsed using sterile distilled water for three times before culture and further air-dried in the laminar air flow hood.

2.2 Plant regeneration from sterile nodal explants

Sterile nodal explants of *C. nutans*were inoculated into jam jars containingMS medium supplemented with four different concentration of hormone BAP (0.5, 1.0, 1.5 and 2.0 mg/L). Sampleswere placed in growth chamber at photoperiod condition (12 h light, 8 h dark) with temperature at 25° C. The observation on plant growth and regeneration were continuously done within 8 weeks and data were analyzed.

2.3 Subculture

To maintain the plantletnourished with enough nutrient, the explants were sub-cultured in a fresh medium consisting of MS medium supplemented with BAP.

2.1 Statistical analysis

The results of the study were analysed by using the Statistical Package for the Social Sciences (SPSS) Enterprise IBM SPSS version 20 for Window 7. One–way ANOVA test was applied to test at 5% less than level of significance which is ($P < 0.05$) in order to determine the variation between means of the parameters that were tested.

3.0 Results and Discussion

3.1 Number of leaves from a plantlet

Five different media treatments were applied to study the influence of BAP on the number of leaves after successfully regenerated from nodal explants.

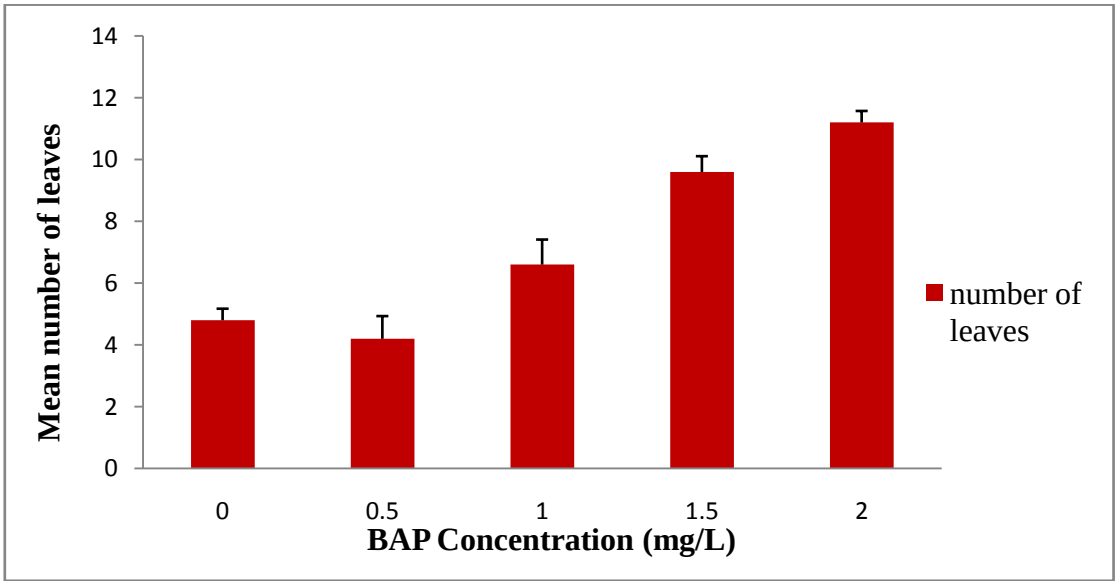

Figure 1: Mean numbers of leaves with different concentration of BAP in MS basal media

The numbers of leaves were recorded after 8 weeks of observation. As shown in Figure 1, the mean number of leaves of 0.5 mg/L BAP concentration was lower (4.20 ± 0.837) compared to the mean number of control. Overall observation from leaves generation showed that the mean number of leaves steadily increase from BAP concentration of 0.5 mg/L until 2.0 mg/L. BAP concentration that able to generate the highest number of leaves was 2.0 mg/L (11.2 ± 0.374). Type ofcytokininandconcentration aretwo key factors for successful *in vitro* multiplication and BAP is one of the effective hormones in promoting proliferation of plant tissue culture. The

plant hormones applied in this study had a significant influence on the successful production of plantlets with high number of leaves.

Between 7 to 8 weeks, it was observed that the leaves started to fall within the culture. The leaves fall inconsistently in each of the BAP concentration treatment. Leaf is the organ that characterizes plants as autotrophs because they fix carbon using light energy and initiate their life as leaf primordial. During its development and growth, they become photosynthetically competent and accumulate nutrients. Leaves then enter the senescence stage, followed by their death and fall from the plant (Woo *et al*., 2013). Senescence is one form of programmed cell death which refers to a proactive, controlled initiation of cell death which is a normal process for growth and development.

Statistical one-way analysis of variance (ANOVA) obtained from the SPSS shows that there is significance difference between the groups ($P < 0.05$) on the number of leaves production. The significance difference indicated that the use of BAP hormone is relevant in manipulating the number of leaves in plant regeneration study.

3.2 Plantlet's Roots Number and Length

Based on the root formation, it can be seen that the nodal explants emerged on the 5^{th}week and after 8^{th}weeks; the number and the length of roots were measured and recorded. The formation of roots in *C. nutans*explants was observed only in the medium containing 2.0 mg/L BAP concentration. Based on the results obtained from the statistical analysis, there was a significance difference between treatments on the number of roots and length of roots ($P<0.05$) with 2.800 ± 0.374 and 5.040 ± 0.443 cm respectively. The rooting of the explants probably related to the increasing of BAP concentration in the medium. Thus, these explants rely on the presence of BAP for root formation. As reported by Taiz and Zeiger (2004),cytokinin do participate in regulation of many plants processes such as the root formation.

3.3 Height of explants

The trend for height of explants in *C. nutans*plants regeneration shows steadily increase by increasing the BAP concentration as shown in Figure 2.

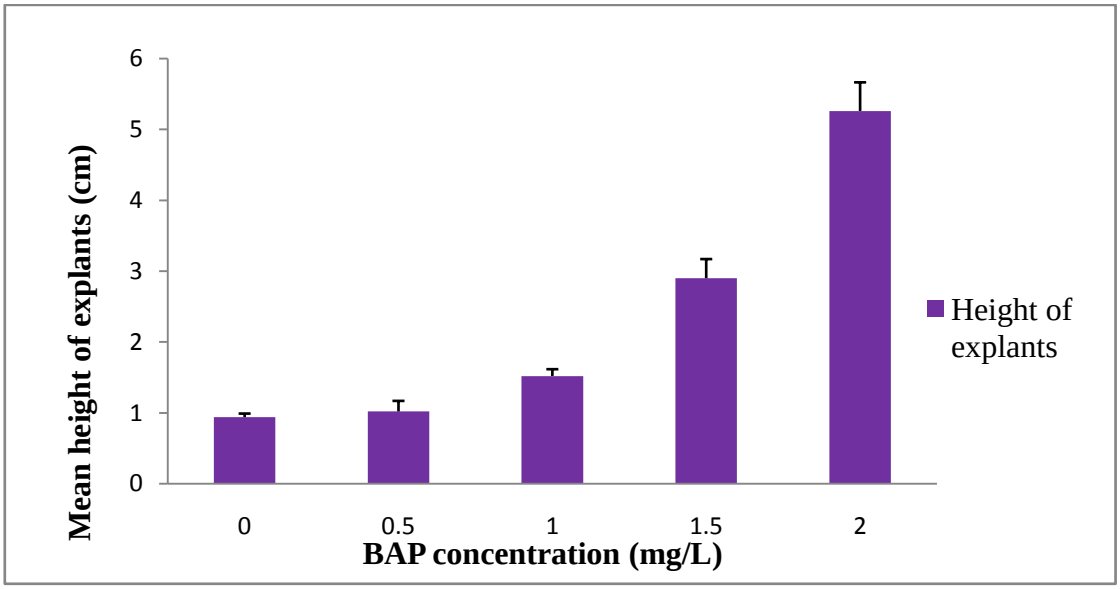

Figure 2: The mean height of explants with different concentration of BAP in MS basal media

This shows that, BAP supplemented hormone in the medium did affect the height of explants. There is a significant difference between all the treatments concentration ($P<0.05$). The highest mean value is 5.260 ± 0.404 cm (2.0 mg/L), meanwhile the lowest mean value is 0.94 ± 0.114 cm (0 mg/L).

4.0 Conclusion

Based on the data obtained, it can be concluded that the explants used mercury chloride methods in order to be free from contamination and the explants were able to surviveand regenerate in full strength of MS media without supplemented with any hormone. However, it can regenerate and survive better with medium culture that supplemented with BAP concentration. Overall of media tested with BAP, it can be concluded that MS media supplemented with 2.0 mg/L is the best concentrationfor*C. nutans*plant regeneration study. It is recommended to increase the BAP concentration in regeneration and to increase the number of parameters used in this study in order to make sure the accuracy of the data more reliable and accurate. Besides that, the variety of cytokinin should be tested in regeneration to observed the best hormone cytokinin which able to regenerate in minimum of time with maximum regeneration and higher survivability.

5.0 References

Bihua, C., Juan, Z., Wenbin, Z., Cui, Z., and Yumei, X. (2015).The rapid propagation technique of the medicinal plant *Clinacanthusnutans*by tissue culture.New York Sciences Journal.*8(2),* 23 – 27.

Purkayastha, J., Sugla, T., Paul, A., Solleti, S. and Sahoo, L. (2007). Rapid *in vitro* multiplication and plant regeneration from nodal explants of *Andrographispaniculata*: a valuable medicinal plant. The Society for *in vitro* Biology. 44, 442 – 447.

Ray, B.P., Hassan, L. and Nasiruddin, K.M., (2011). *In vitro* regeneration of Brinjal*(Solanummelongena L.*). *36(3),* 397-406

Taiz, L and Zeiger, E. (2004).Plant physiology. Porto Alegre: Artmed. 449 – 484.

Thorpe, A. T. (2005). History of plant tissue culture.In Plant Cell Culture Protocols. Victor, M. L and Felipe, V. (ed). Human Press Inc, Totowa, New Jersey. 9-33 pp

Veeresham, C. and Chitti, P. (2013).Therapeutic agents from tissue cultures of medicinal plants.*Natural Products Chemistry & Research, 1(4),* 1-5

Woo H. R., kim, H. J., Nam, H. G., and Lim, P. O. (2013). Plant leaf senescence and death- regulation by multiple layers of control and implications for aging in general. Journal of cell sciences *126(21),* 4823 – 4833

Ying, N.L. (2013). Establishment of axenic explants and callus culture of *Clinachanthusnutans*(RumputBelalai Gajah).*Plant Resources Science and Management*, 14-24.